

QUANTITATIVE DETERMINATION OF HUMAN CONNECTIVE TISSUE GROWTH FACTOR (CTGF)

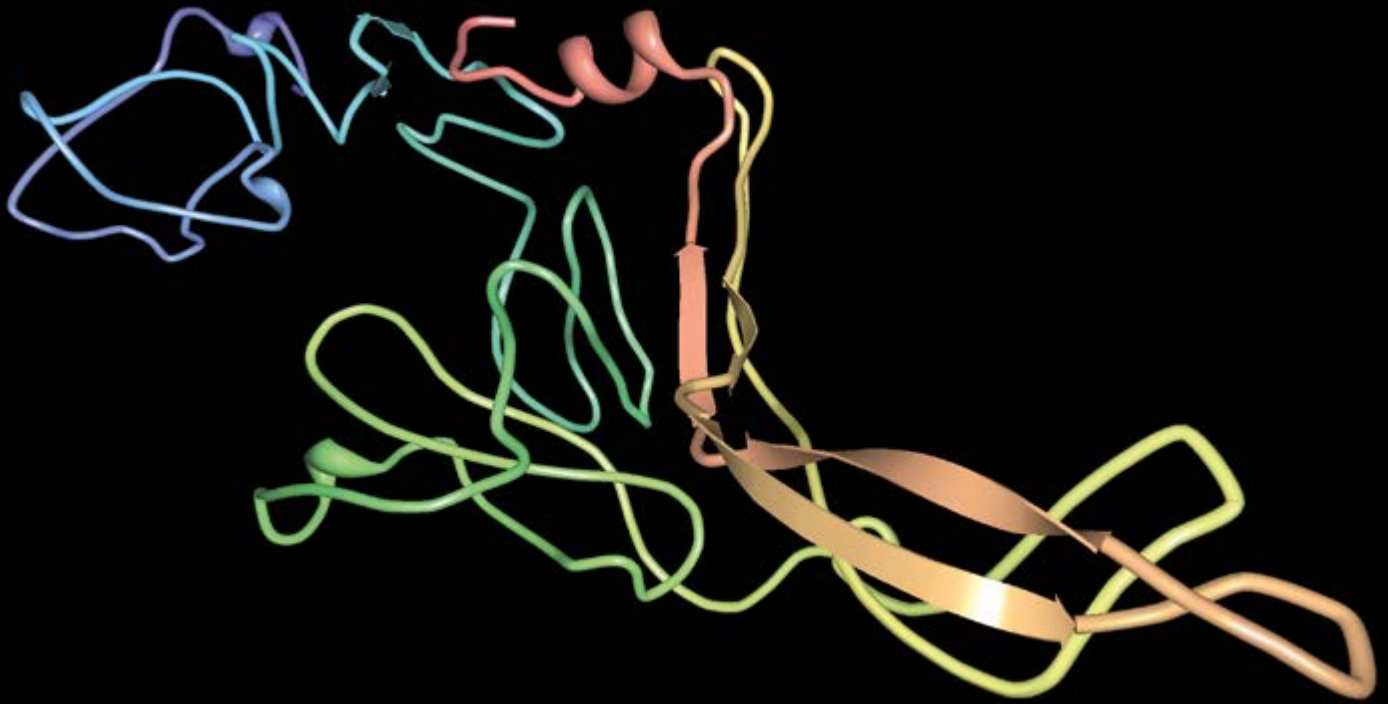
NEW PRODUCT

Human Connective Tissue Growth Factor ELISA

- › High sensitivity (0.02 ng/ml)
- › Excellent analytical characteristics
- › Validated for human plasma (citrate, heparin) and urine (and/or serum)
- › Preliminary population data

**CARDIOVASCULAR DISEASE
CORONARY ARTERY DISEASE
RENAL DISEASE · PULMONARY DISEASE
LIVER · RHEUMATOID ARTHRITIS (RA)
DIABETOLOGY · ONCOLOGY · APOPTOSIS
FIBROSIS · WOUND HEALING**

HUMAN CONNECTIVE TISSUE GROWTH FACTOR ELISA



Introduction

Connective Tissue Growth Factor (CTGF, CCN2, HCS24, IGFBP8, NOV2) is a heparin-binding glycoprotein with a molecular weight of 36-38 KDa, composed of 343-349 amino acid residues and belonging to the CCN family. The CCN family of proteins is a complex family of multifunctional proteins containing six members designated CCN1 to CCN6 [1, 2]. The CTGF protein consists of four conserved domains (insulin-like growth factor; von Willebrand factor type C repeat; thrombospondin type-I repeat module and cysteine-knot-containing module) which can interact with different extracellular proteins including growth factors, integrins or proteoglycans. Due to its modular structure, the CTGF is associated with many biological processes such as angiogenesis, chondrogenesis (cartilage regeneration), osteogenesis, tissue repair or proliferation and it is critically involved in fibrotic disease and several forms of cancer [3].

Under normal circumstances and physiological conditions, CTGF is present at low or undetectable levels. Despite the low expression, CTGF plays an important role in many physiological processes such as formation and growth of the skeletal system, proper formation of islets in the pancreas and hair follicle development in the skin. Recent studies also suggested the involvement of CTGF in the development of other tissues including tooth, eye and platelet [4].

High CTGF expression is mainly associated with pathological conditions. Connective tissue growth factor is well known as a molecule that is involved in inflammation, wound healing and fibrosis and mediates the development of fibrotic disorders in a variety of tissues and organs. An elevated level of CTGF was observed in individuals with fibrosis in the heart, lung, liver, kidney and skin [5-7]. In addition to fibrosis, aberrant CTGF expression has also been observed with many types of malignancies, diabetic nephropathy and retinopathy, arthritis, and cardiovascular diseases [8-10]. These findings indicate that increased levels of CTGF in serum, plasma and/or urine can be used as a useful biomarker of relevant disease and, in combination with other organ-specific biomarkers, could increase diagnostic and prognostic accuracy and clinical judgment [11].

Extensive investigation on Connective Tissue Growth Factor (CTGF) over decades has revealed its novel molecular action and functional properties. Due to its modular structure, CTGF binds to numerous proteins; it is associated with many biological processes and today it is not considered a classical growth factor, but rather a modulator of activities of other growth factors [10].

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BioVendor Human Connective Tissue Growth Factor ELISA (RD191035200R)

Intended use

The RD191035200R Human Connective Tissue Growth Factor ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human connective tissue growth factor (CTGF).

- The total assay time is less than 3.5 hours
- The kit measures total CTGF in plasma (EDTA, citrate, heparin) and urine and/or serum
- Assay format is 96 wells
- Standard is recombinant protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

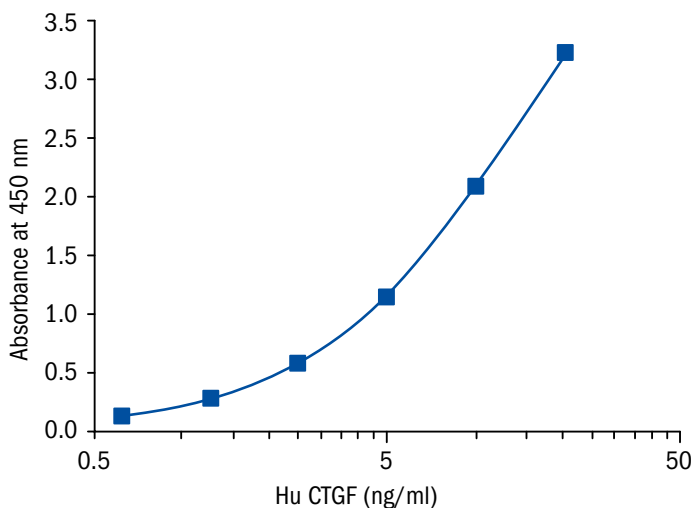
Clinical application

- Cardiovascular disease
- Coronary artery disease
- Renal disease
- Pulmonary disease
- Liver
- Rheumatoid arthritis (RA)
- Diabetology
- Oncology
- Apoptosis
- Fibrosis
- Wound healing

HUMAN CONNECTIVE TISSUE GROWTH FACTOR ELISA CAT. NO.: RD191035200R	
Assay format	Sandwich ELISA, Biotin-labelled antibody, 96 wells/kit
Samples	plasma (EDTA, citrate, heparin) and urine and/or serum
Standards	0.63 to 20.0 ng/ml
Limit of detection	0.02 ng/ml

Test principle

In the BioVendor Human Connective Tissue Growth Factor ELISA, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-human CTGF antibody. After 60 minutes incubation and a washing, biotin-labelled polyclonal anti-human CTGF antibody is added and incubated with captured CTGF for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of CTGF. A standard curve is constructed by plotting absorbance values against concentrations of CTGF standards, and concentrations of unknown samples are determined using this standard curve.



HUMAN CONNECTIVE TISSUE GROWTH FACTOR ELISA

Precision

Intra-assay (Within-Run) (n=8)

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	21.2	0.62	2.9
2	36.6	1.05	2.9

Inter-assay (Run-to-Run) (n=7)

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	22.1	0.91	4.1
2	39.5	1.79	4.5

Spiking recovery

Serum samples were diluted, spiked with different amounts of human CTGF and assayed.

Sample	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
Serum 1	13.4	-	-
	20.2	19.7	102.6
	26.7	25.9	103.1
	37.8	38.4	98.5
Serum 2	20.3	-	-
	31.9	32.8	97.1
	43.0	45.3	94.9
	67.8	70.3	96.4

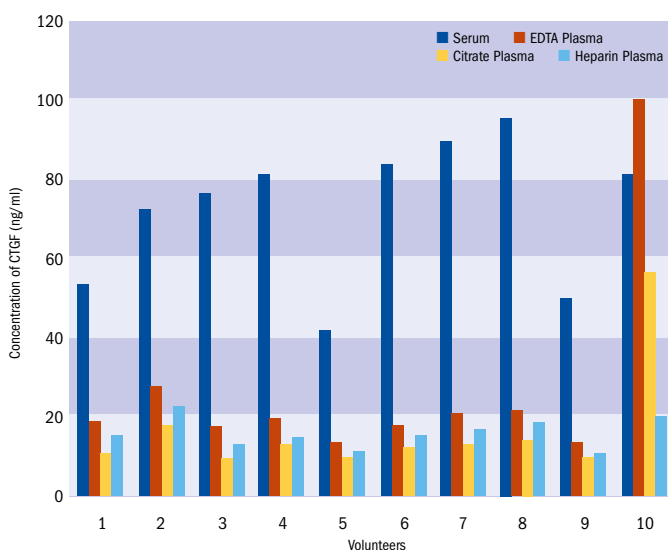
Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
Serum 1	-	38.0	-	-
	2x	18.2	19.0	95.6
	4x	8.57	9.50	90.2
	8x	4.17	4.75	87.6
Serum 2	-	69.4	-	-
	2x	33.9	34.7	97.8
	4x	15.5	17.4	89.0
	8x	7.33	8.68	84.5

Effect of sample matrix

Citrate and heparin plasma and serum samples were compared to respective EDTA plasma samples from the same 10 individuals. Results are shown below:



Summary of protocol

- Reconstitute Master Standard and prepare set of standards
- Dilute samples (10x)
- Add 100 µl standards, samples and IQCs
- Incubate at RT for 1 hour / 300 rpm
- Wash plate 5 times
- Add 100 µl Biotin Labelled Antibody
- Incubate at RT for 1 hour / 300 rpm
- Wash plate 5 times
- Add 100 µl Streptavidin-HRP Conjugate
- Incubate at RT for 30 min / 300 rpm
- Wash plate 5 times
- Add 100 µl Substrate Solution
- Incubate at RT for 10 min
- Add 100 µl stop solution
- Read absorbance and calculate results

Crossreactivity

The antibodies used in this ELISA are specific to human CTGF.

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Preliminary Population Data

The following results were obtained when serum samples from 155 unselected donors (89 men + 66 women) 20 - 65 years old were assayed with the BioVendor Human Connective Tissue Growth Factor ELISA in our laboratory.

Age and Sex Dependent Distribution of CTGF

Sex	Age (years)	n	Mean CTGF (ng/ml)	Median CTGF (ng/ml)	SD CTGF (ng/ml)	Min. CTGF (ng/ml)	Max. CTGF (ng/ml)
Male	20-29	18	46.1	59.1	15.1	26.7	82.5
	30-39	26	45.7	44.6	14.0	22.7	93.3
	40-49	31	47.1	45.9	14.0	22.3	76.7
	50-65	14	43.2	44.9	6.83	29.8	51.9
Female	20-29	12	50.0	48.4	16.4	25.7	81.4
	30-39	26	43.3	40.9	12.7	19.7	82.2
	40-49	20	45.0	41.2	11.8	28.8	66.7
	50-61	8	43.4	40.4	9.8	31.0	66.1

Related products

- RD172035100-HEK Connective Tissue Growth Factor Human HEK293
- RD172035100 Connective Tissue Growth Factor Human *E. coli*

References

1. Gressner, O.A. and A.M. Gressner, Connective tissue growth factor: a fibrogenic master switch in fibrotic liver diseases. *Liver Int*, 2008. 28(8): p. 1065-79.
2. Takigawa, M., et al., Role of CTGF/HCS24/ecogenin in skeletal growth control. *J Cell Physiol*, 2003. 194(3): p. 256-66.
3. Shi-Wen, X., A. Leask, and D. Abraham, Regulation and function of connective tissue growth factor/CCN2 in tissue repair, scarring and fibrosis. *Cytokine Growth Factor Rev*, 2008. 19(2): p. 133-44.
4. Kubota, S. and M. Takigawa, Cellular and molecular actions of CCN2/CTGF and its role under physiological and pathological conditions. *Clin Sci (Lond)*, 2015. 128(3): p. 181-96.
5. Leask, A. and D.J. Abraham, All in the CCN family: essential matricellular signaling modulators emerge from the bunker. *J Cell Sci*, 2006. 119(Pt 23): p. 4803-10.
6. Jun, J.I. and L.F. Lau, Taking aim at the extracellular matrix: CCN proteins as emerging therapeutic targets. *Nat Rev Drug Discov*, 2011. 10(12): p. 945-63.
7. Kular, L., et al., The CCN family: a new class of inflammation modulators? *Biochimie*, 2011. 93(3): p. 377-88.
8. Ellina, O., et al., Extracellular matrix-associated (GAGs, CTGF), angiogenic (VEGF) and inflammatory factors (MCP-1, CD40, IFN- γ) in type 1 diabetes mellitus nephropathy. *Clin Chem Lab Med*, 2012. 50(1): p. 167-74.
9. McLennan, S.V., M. Abdollahi, and S.M. Twigg, Connective tissue growth factor, matrix regulation, and diabetic kidney disease. *Curr Opin Nephrol Hypertens*, 2013. 22(1): p. 85-92.
10. Klaassen, I., et al., The role of CTGF in diabetic retinopathy. *Exp Eye Res*, 2015. 133: p. 37-48.
11. Dendooven, A., et al., Connective tissue growth factor (CTGF/CCN2) ELISA: a novel tool for monitoring fibrosis. *Biomarkers*, 2011. 16(4): p. 289-301.

Contact Information



BioVendor – Laboratorni medicina a.s.
Karasek 1767/1, 621 00 Brno, Czech Republic
Phone: +420 549 124 185, Fax: +420 549 211 460
E-mail: info@biovendor.com

BioVendor GmbH
Otto-Hahn-Straße 16, 34123 Kassel, Germany
Phone: +49 6221 4339 100, Fax: +49 6221 4339 111
E-mail: infoEU@biovendor.com

› www.biovendor.com

BioVendor GesmbH
Nußdorfer Straße 20/10, 1090 Vienna, Austria
Phone: +43 1 890 9025, Fax: +43 1 890 9025-15
E-mail: infoAustria@biovendor.com

BioVendor, LLC
128 Bingham Rd., Suite 1300, Asheville, NC 28806, USA
Phone: +1-800-404-7807, Phone: +1-828-575-9250
Fax: +1-828-575-9251, E-mail: infoUSA@biovendor.com